Incorporation of *Himanthalia elongata* seaweed to enhance the phytochemical content of breadsticks using Response Surface Methodology (RSM)

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Abstract

Optimization of incorporating seaweed into breadsticks was carried out using response surface methodology (RSM). Ten formulations of breadsticks were processed by varying concentrations of seaweed (X_1 = 5 to 15% of overall flour concentration) and white flour (X_2 = 10 to 30% of overall flour concentration) using a central composite design. The remaining flour concentrations were comprised of wholemeal flour. Predicted models were found to be significant (P < 0.05) for total phenolic content (TPC), DPPH radical scavenging activity, texture and color. Predicted values for each of the responses were in good agreement with the experimental values. Seaweed concentration had most significant effect on phytochemical constituents of the breadsticks with TPC and DPPH activity maximized when 17.07% *H. elongata* was incorporated into the flour (P < 0.05). An acceptable edible texture and color of breadsticks was also achieved at this concentration. Multiple response optimization demonstrated that phytochemical content of *H. elongata* breadsticks may be maximized with dried seaweed and white flour concentrations of 17.07 and 21.89%, respectively, in the total flour. Total dietary fiber increased from 4.65 to 7.95% in the optimized sample, representing a 43.65% increase as compared to the control (P < 0.05). A sensory panel evaluated the acceptability of the seaweed breadsticks, as compared to the control, in terms of aroma, color, texture, taste and overall acceptability. There was no significant difference (P > 0.05) between the seaweed breadsticks and the control which shows that such fiber-rich seaweed bakery products are acceptable to consumers and have potential of increasing seaweed consumption among non-seaweed consumers.

Introduction

Marine food, due to its phenomenal biodiversity is a treasure house of many novel healthy food ingredients and biologically active compounds such as those found in seaweeds. Despite having so many health benefits, marine functional foods have been underexploited for food purposes. Bakery products are widely consumed throughout the world and are the best sources of incorporating marine functional ingredients and reaching the targeted population (Kadam and Prabhasankar, 2010). Bread is an excellent product in which incorporation of 'nutraceuticals' is attempted. One of the latest enrichments has been the addition of omega-3 PUFA to improve essential fatty acid intake. In Europe, consumption of bread enriched with omega-3 PUFA is steadily increasing because Europeans recognise the healthy component of such products. Therefore, the near future for nutrition could potentially include extending the use of breads as vehicles for different micronutrients (Kadam and Prabhasankar, 2010).

Seaweed contains a significant amount of soluble polysaccharides, and has potential function as dietary fiber. The seaweed polysaccharides possess a higher Water Holding Capacity (WHC) than cellulosic fibers. There is an interest in seaweed hydrocolloids for human nutrition as they can act as dietary fiber since their physiological effects are closely related to their physicochemical properties such as solubility, viscosity, hydration, and ion-exchange capacities in the digestive tract (Lahaye and Kaeffer, 1997). Dietary fiber (DF) is the edible portion of plants (or analogous carbohydrates) which is resistant to digestion and adsorption in the human small intestine with complete or partial fermentation in the large intestine (Gelroth and Ranhotra, 2001). The term DF comprises polysaccharides, oligosaccharides and associated plant compounds (AACC, 2001).

Brown seaweeds are known to contain more bioactive components than red or green seaweeds (Seafoodplus, 2008). Some of the bioactive compounds identified in brown seaweeds include phylopheophylin, phlorotannins, fucoxanthin and various other metabolites (Hosakawa *et al.*, 2006). Such antioxidants from natural sources can be added to products as an ingredient to increase the quality and shelf-life which also considerably enhances the

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consumer preference (Farag *et al.*, 2003).

Development of functional foods is currently one of the most intensive areas of food product development worldwide. Product optimization is an effective strategy to accomplish successful development of the product with respect to a number of attributes. If a food product cannot be re-engineered or modified to fulfill consumer desires and demand for the product, it will not succeed (Robinson, 2000). The present study aimed to identify a food-based application for dried edible Irish seaweed in order to encourage consumption amongst non-seaweed eaters. The idea was to scientifically evaluate and improve the quality and nutritional content of a bakery product upon the incorporation of seaweeds. Wheat is the principal cereal used in the preparation of a variety of bakery products, however there is a current trend to move away from white breads towards whole grains such as whole meal flour. Therefore in the present study, white flour concentration was also varied and the overall flour consisted of varying levels of dried seaweed, white and wholemeal flours. The main objective was to optimize the dried seaweed and white flour concentrations in the development of a new bakery based functional product and to investigate its effect on the phytochemical content of breadsticks.

Materials and Methods

Chemicals

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate (Na_2CO_3) and total dietary fiber kit were purchased from Sigma Aldrich Chemie (Steinheim, Germany).

Seaweed material

H. elongata was purchased from Quality Sea Veg., Co Donegal, Ireland. The seaweeds were collected in October 2011 and stored at 4°C until further use.

Preparation of samples

H. elongata was washed thoroughly with tap water to remove epiphytes and salt, dried with absorbent paper and then cut into 3 cm long pieces before dehydration.

Dehydration procedure

Drying temperature and time was decided based on results of our previous kinetic experiments (Gupta *et al.*, 2011). Seaweed samples (5 g) were placed on a drying tray in a single layer. Drying of seaweed was carried out in a drier (Innova 42, Mason Technology, Ireland) at 40°C air drying temperature over a period of 24 hours. Air velocity was 2.0 ± 0.1 m s^{-1} measured

with VWR Enviro-meter digital anemometer (VWR, Ireland). The dried seaweed was then ground into a fine powder using a blender (Rotor, Germany).

Experimental design

To investigate the effect of factors (seaweed and white flour concentration) on phytochemical constituents, color and texture of breadsticks, a central composite design with two factors was utilised. The central composite design was applied using STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA, USA). The total number of experiments generated from the software with two factors was 10 (= $2^k + 2k + 2$), where k is the number of factors. Eight experiments were augmented with two duplicates at the centre points. The level of codes for the independent variables are presented in Table 1. The design matrix and variable combinations of seaweed and white flour concentrations in experimental runs are shown in Table 2. The independent variable concentrations applied in the response surface methodology (RSM) study (Seaweed 5 - 15% and white flour 10 - 30%) were percentage of the of the overall flour concentration, with wholemeal flour making up the remaining quantity up to 100%. Therefore as a percentage of the overall mix of 411 g, these values consisted of 1.82 - 10.33 and 3.65 - 20.67% (seaweed and white flour, respectively).

Table 1. Level of codes for independent variables used in the central composite design

Independent variables	Symbol	-2	-1	0	+1	+2
Seaweed concentration (%)*	X_1	2.93	5	10	15	17.07
White flour concentration (%)*	X_2	5.86	10	20	30	34.14

*Percentage of overall flour concentration (100%) with the remaining flour consisting of wholemeal

Table 2. Design matrix and variable combinations in experimental runs

Experiment	Seaweed concentration (%)*	White flour concentration (%)*
1	15.00	10.00
2	10.00	20.00
3	5.00	30.00
4	10.00	20.00
5	17.07	20.00
6	10.00	5.86
7	5.00	10.00
8	2.93	20.00
9	10.00	34.14
10	15.00	30.00

*Percentage of overall flour concentration (100%) with the remaining flour consisting of wholemeal

Experimental data from the central composite design was analysed and fitted to a polynomial regression model below:

$$Y = \beta_0 + \beta_1 \chi_1 + \beta_2 \chi_2 + \beta_{11} \chi^2_1 + \beta_{22} \chi^2_2 + \beta_{12} \chi_1 \chi_2 \quad \text{Eq. 1}$$

Where; Y is response calculated by the model: β_0 is a constant and β_i, β_{ii} and β_{ij} are linear, squared and interaction coefficients, respectively.

The adequacy of the model was evaluated by the lack of fit, coefficient of determination (R^2) and the Fisher's test value (*F*-value) obtained from the analysis of variance (ANOVA) generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level ($\alpha = 0.05$). Three-dimensional response surface plots and contour plots were generated by keeping one response variable at its optimal level and plotting that against two factors (independent variables).

A multi-response analysis of the response surface design was performed using the desirability approach to optimize seaweed and white flour concentrations. The desirability function is an approach for solving the problem of optimization of several responses and is applied when various responses have to be considered at the same time and it is necessary to find optimal compromises between the total numbers of responses taken into account. This methodology is based on first constructing a desirability function for each individual response, and then it is possible to obtain the overall desirability.

Seaweed breadstick preparation

Seaweed and flour blends were prepared by the replacement method according to the RSM experiment. The percentages of seaweed and white flour from the RSM (Table 2) are based on percentages of overall flour in the mix (flour consisted of 60.79% of the mix), with wholemeal flour comprising the remaining component of the mix. The concentrations of ingredients for each of the experiments can be seen in Table 3. Firstly, the yeast was dissolved in the water and added to the dry ingredients (except seaweed). The ingredients were mixed at slow speed for 2 min, then at medium speed for 4 min (Hobard A120 mixer, Hobard MFG Co. Ltd, London, UK). Seaweed was then added and mixed again for a further 2 min. The dough was placed on trays and left to develop for 45 min then moulded into breadstick shapes by hand and proofed in a dough proofer (Sveba Dahlen, Sveba Dahlen, Fristan, Sweden) at 33°C, 78% RH for 40 min. The breadsticks were then baked in an oven (Sveba Dahlen, DC 44, Sveba Dahlen, Fristan, Sweden) at 210°C for 20 min with 10 seconds of steam at the beginning.

Extraction of phytochemicals

Seaweed and breadstick samples (5 g) were powdered in liquid nitrogen using a mortar and pestle, then extracted with 50 ml of methanol (60%) under nitrogen atmosphere for 2 hours as described by Cox *et al.* (2010).

Total phenolic content

The total phenolic concentration was measured using the Folin-Ciocalteau method as outlined by Cox *et al.* (2012). The total phenolic contents were expressed as mg gallic acid equivalent per 100 gram dry basis (db) (mg GAE/100 g db).

DPPH radical scavenging activity

Free radical scavenging activity was measured by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Jaiswal *et al.* (2011).

Texture evaluation

Shear tests were performed using an Instron Universal Testing Machine (Model 4301, Canton MA, USA) supported with Bluehill 2 version 2.14 analysis software for materials testing. A Warner Bratzler cutter was used in the shear tests. An aluminium plate with dimensions of 10 x 6 cm^2, thickness of 1.3 cm and with an opening of 3 mm in the centre was supported in the Instron base. Breadstick samples (5 g) were sheared at a speed of 200 mm/min. The cutting implement was allowed to travel the depth of the seaweed, cutting through the sample and seaweed hardness was defined as the peak of force-deformation curve recorded in Newtons per mm (N/mm). Ten replications of each sample were carried out.

Color measurement

At specified experimental times (Table 2), breadsticks (original 5 g FW) underwent color analysis using a colorimeter (CIE Lab ColorQuest XE). The colorimeter was calibrated against a standard white reference tile ($L^* = 93.97$; $a^* = -0.08$ and $b^* = 1.21$). The color values were represented on the CIE color scales in terms of L^* (lightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness). From these values, total color change from fresh (ΔE) was calculated according to the following equation:

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad \text{Eq. 2}$$

Where; L^*_0, a^*_0 and b^*_0 are the readings at time zero and L^*, a^* and b^* are the individual readings at each drying time.

Total dietary fiber

Total dietary fiber (TDF) was determined by Sigma analysis kit (Sigma-Aldrich, Inc., USA) based

Table 3. Design of experiments for seaweed breadsticks

Experiment	Seaweed (%)	White flour (%)	Wholemeal flour (%)	Salt (%)	Butter (%)	Yeast (%)	Water (%)
1	9.12	6.08	45.59	1.21	1.21	2.13	34.65
2	6.08	12.16	42.55	1.21	1.21	2.13	34.65
3	3.04	18.24	39.51	1.21	1.21	2.13	34.65
4	6.08	12.16	42.55	1.21	1.21	2.13	34.65
5	10.33	12.16	38.30	1.21	1.21	2.13	34.65
6	6.08	3.65	51.06	1.21	1.21	2.13	34.65
7	3.04	6.08	51.67	1.21	1.21	2.13	34.65
8	1.82	12.16	46.81	1.21	1.21	2.13	34.65
9	6.08	20.67	34.04	1.21	1.21	2.13	34.65
10	9.12	18.24	33.43	1.21	1.21	2.13	34.65

on AOAC method 991.43. Samples were cooked at 100ºC with heat stable α-amylase to initiate gelatinization, hydrolysis and depolymerisation of starch. The samples were incubated at 60ºC with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose). The samples were then treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerised protein and glucose. The residue was filtered, washed, dried and weighed. One duplicate was analysed for protein and the other was incubated at 525ºC to determine ash. The TDF was determined as the weight of the filtered and dried residue less the weight of the protein and ash.

Sensory characteristics

The sensory acceptance test was conducted in a standardized sensory test room (ISO 8589, 2007). Untrained panelists (n = 20) were recruited from staff and students of the Dublin Institute of Technology using a five-point hedonic scale. Samples (20 g) were served on white paper plates with random three-digit numbers and water at room temperature was provided for mouth-rinsing between samples. The panelists were asked to assign scores for aroma (maximum of 5), appearance (maximum of 5), texture (maximum of 5), flavour (maximum of 5) and overall acceptability of the product (maximum of 5), where 5 was "like extremely" and 1 was "dislike extremely". The overall quality (maximum of 25) was computed by combining scores of all five attributes.

Statistical analysis

All experiments were carried out in triplicate and replicated at least twice. Data from the central composite design were subjected to a second-order multiple regression analysis using least-squares regression to obtain the parameter estimated for the mathematical model. The regression analysis

and analysis of variance (ANOVA) were performed with the STATGRAPHICS Centurion XV software (StatPoint Technologies, Inc., Warrenton, VA). Differences were considered statistically significant when $P < 0.05$.

Results and Discussion

Statistical analysis of results obtained by experimental design

The effect of a range of drying temperatures on the drying kinetics and phytochemical constituents of *H. elongata* was investigated and results showed that drying was optimized at 40°C and therefore these drying conditions were applied in the current study (Gupta *et al*., 2011). The rationale behind adding seaweed to breadsticks was based on the fact that bakery products are widely consumed; therefore addition of *H. elongata* would widen the consumer base and would further improve the nutraceutical properties of this product. Dried seaweed is also a convenient and cost effective ingredient as drying reduces the volume thus lowering transport costs and therefore can be considered a viable ingredient to add value to existing products.

Preliminary experiments were carried out in order to determine the maximum levels of seaweed which could be added to the breadsticks with respect to texture and flavour. Higher seaweed concentrations ($\geq$ 20%) led to unacceptable end products as the baked product was quite tough and difficult to chew. Once the maximum level of seaweed was established at 15%, RSM was applied. In this study, ten experiments were performed to determine the optimum concentrations of seaweed and flour blends required to maximize the phytochemical level in breadsticks. The effects of independent variables (seaweed and white flour concentrations) for each of the response variables (TPC, DPPH, texture and color) are presented in Table 4.

Table 4. Two-way ANOVA for the independent variables on the response of total phenolic content, DPPH, texture and color of seaweed breadsticks

Source	Total phenolic content		DPPH		Texture		Color	
	F-Ratio	P-value	F-Ratio	P-value	F-Ratio	P-value	F-Ratio	P-value
X_1	762.40	0.0000	66.82	0.0012	2020.32	0.0000	1.50	0.2874
X_2	0.11	0.7548	0.12	0.7464	2.17	0.2145	0.22	0.0345
X_1*X_1	3.13	0.1515	4.65	0.0973	74.44	0.0010	9.93	0.0345
X_1*X_2	0.09	0.7760	0.06	0.8192	0.00	0.9829	2.06	0.2242
X_2*X_2	0.03	0.8812	2.11	0.2203	0.23	0.6579	3.40	0.1390

R^2 values: 0.9948 (total phenolic content), 0.9973 (DPPH), 0.9981 (texture) and 0.7780 (color)

The models for each of the responses were analyzed separately before overall optimum seaweed

and flour concentrations for the breadstick recipe were determined. Predicted and experimental values for each of the responses are presented in Table 5 and were in good agreement with the experimental values. Response surface plots were generated to illustrate the effects of blanching time and temperature on each of the responses (Figure 1a-d).
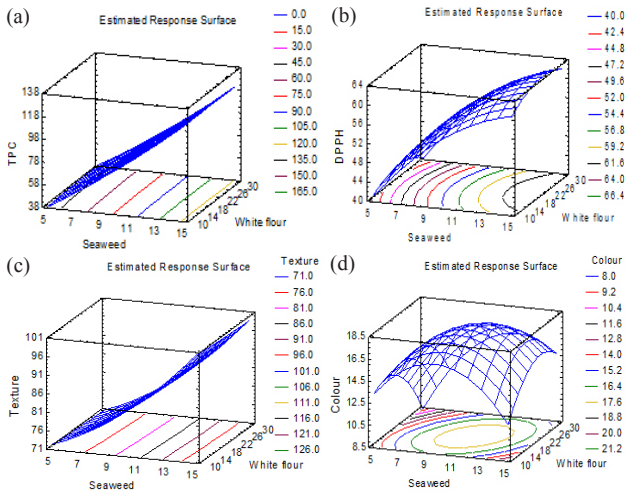

Figure 1. Response surface plots showing effects of seaweed and white flour concentrations (%) on (a) the total phenolic content (GAE/100 g db), (b) DPPH radical scavenging activity (%), (c) texture (N/mm) and (d) color (ΔE) of seaweed breadsticks

Effects of process variables on total phenolic content

Experimental results for total phenolic content (TPC) were fitted to a full quadratic second order polynomial equation and the model obtained for TPC of the breadsticks was:

$$Z = 3.77979 + 5.72532^* X_1 + 0.305353^* X_2 + 0.140273^* X_1^2 - 0.0129^* X_1^* X_2 - 0.00315601^* X_2^2 \quad \text{Eq. 3}$$

(See Table 1 for definitions of X_1 and X_2). In order to determine the significance of the model, ANOVA was carried out on the data. The *F*-value for seaweed concentration (X_1) was high (762.40) indicating that this factor was highly significant (Table 4). All other interaction factors and white flour concentration (X_1) had low F-values which suggest that TPC had mainly resulted from the addition of seaweed. The model explained 99.48% (R^2 of 0.9948) of the variation in TPC which is quite significant. This indicates that only 0.52% of the variation in TPC was due to factors not included in the model.

The P-values were used to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the P-value, the larger the significance of the corresponding coefficient is. P-values indicated that, among the test variables and their interactions,

X_1 (seaweed concentration) was highly significant (P < 0.05) but all other factors; X2 (white flour concentration), $X_1^*X_1$ (seaweed concentration × seaweed concentration), $X_1^*X_2$ (seaweed concetration × white flour concentration) and $X_2^*X_2$ (white flour concentration × white flour concentration) were insignificant model terms with P-values > 0.05.

The polynomical response models were expressed as three-dimensional (3D) surface plots to better visualise the relationship between the seaweed and white flour concentrations as independent variables and phytochemical properties as response variables. The response plot (Fig. 1a) showed that TPC increased sharply with increasing seaweed concentration (P < 0.05), while TPC remained unchanged with increasing white flour concentration as observed in Table 4.

The addition of seaweed to the breadsticks significantly increased the TPC (P < 0.05). An 81.03% increase was seen when the overall flour concentration was substituted with 17.07% seaweed. These results are higher than those reported for other cereal based food products which were incorporated with seaweed. Prabhasankar *et al.* (2009a) studied the influence of adding brown seaweed, *Sargassum marginatum*, to pasta. The TPC in cooked pasta increased from 9 to 13 mg GAE/100 g with 5% addition of the brown seaweed. Although the previous study showed that phenolics leached into processing water, these results are still significantly lower than those of the present study. Comparing with the same seaweed concentration, the results of 5% incorporation of seaweed in breadsticks increased the TPC from 27.67 to 38.99 mg GAE/100 g db which is also higher than that of Prabhasankar *et al.* (2009a).

The breadsticks containing maximum *H. elongata* concentration (17.07%) showed an increase in the TPC from 27.67 to 145.88 mg GAE/100 g db which is an increase of 81.03%, as compared to the control. Prabhasankar *et al.* (2009b) also reported that an addition of 30% *Undaria pinnatifida* seaweed increased the TPC of pasta from 9 - 27 mg GAE/100 g. Again, this is considerably less than obtained in the present study. TPC of bread samples with different percentages of ginger powder were studied by Balestra *et al.* (2011). TPC levels increased from 14.30 to 48.50 GAE/100 g db with 6% addition of ginger powder. This clearly shows that the seaweed breadsticks had higher levels of total phenols compared to that of other nutraceutical cereal based products such as bread and pasta.

Effects of process variables on DPPH radical scavenging activity

The model obtained for the DPPH radical

Table 5. Predicted (Pred.) and experimental (Exp.) values of total phenolic content, DPPH, texture and color of seaweed breadsticks

Experiment No.	Total phenolic content (mg GAE/100g db)		DPPH (%)		Texture (N/mm)		Color (ΔE)	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	118.02	122.02	60.25	60.50	99.36	99.99	11.85	11.75
2	78.99	77.33	52.18	57.08	81.51	81.82	19.17	18.39
3	38.99	40.30	40.44	40.76	70.90	71.14	5.47	8.50
4	75.66	77.33	61.98	57.08	82.12	81.82	17.6	18.39
5	145.88	142.84	65.24	64.46	108.84	108.66	12.79	12.07
6	80.16	75.99	51.36	51.62	83.82	82.86	14.35	14.36
7	34.55	38.01	41.21	40.76	71.05	72.04	12.34	13.29
8	28.11	25.84	35.11	35.32	69.85	69.16	10.96	8.76
9	78.54	77.40	53.69	52.86	81.47	81.56	16.02	13.08
10	119.88	121.74	61.22	62.24	99.17	99.05	12.75	14.73

Values are presented as mean (n = 6).

scavenging activity of the breadsticks was:

$$Z = 13.2787 + 4.76275^* X_1 + 0.92469^* X_2 - 0.1438^* X_1^2 + 0.0087^* X_1^* X_2 - 0.0242^* X_2^2 \quad \text{Eq. 4}$$

There was a significant (P < 0.05) influence of the linear factor of X_1 (seaweed concentration) on the model. The linear factor of X_2 (white flour concentration) and all quadratic factors and interactions $X_1^*X_1$ (seaweed concentration × seaweed concentration), $X_1^*X_2$ (seaweed concetration × white flour concentration) and $X_2^*X_2$ (white flour concentration × white flour concentration) were insignificant model terms with P-values > 0.05 in terms of DPPH radical scavenging activity. This showed that seaweed concentration had the greatest impact on the DPPH radical scavenging activity of the breadsticks which was expected as seaweed exhibit high levels of DPPH radical scavenging activity. The fit of the model was further confirmed by a high coefficient of determination, 0.9973 meaning that 99.73% of the variation in DPPH activity was explained by the model. The response surface plots generated showed that DPPH radical scavenging activity increased with increasing seaweed concentration while the activity remained more or less constant with respect to the effect of white flour concentration (Figure 1b). The lack of significance of the white flour concentration on the DPPH activity of the breadsticks is further confirmed by the circular shape of the contour plots which indicates that the interactions are negligible.

The DPPH radical scavenging activity of the control breadsticks (containing no seaweed) was 34.81%. Replacement of flour with 17.07% seaweed increased the DPPH activity to 65.24%, representing a significant increase of 46.64% in DPPH activity (P < 0.05). Any level of seaweed above 5% significantly increased the DPPH activity of the seaweed breadsticks (P < 0.05). Balestra *et al.* (2011) also found a significant increase in DPPH activity with

the addition of 6% ginger powder to breads (86.75% increase). In seaweed incorporated pasta, it was found that addition of 30% brown seaweed increased the DPPH activity from 6.83 to 9.79% (Prabhasankar *et al.*, 2009a) which is significantly lower than the activity in the present study. In our previous studies, it is reported that dehydration can lead to slight decreases in DPPH activity but thermal processing such as boiling, applied after drying can lead to significant increases in the activity (Cox *et al.*, 2011). It is possible that the temperature upon baking of the breadsticks could also have increased the DPPH radical scavenging activity of extracts from the final product. This indicates that addition of *H. elongata* seaweed to breadsticks would provide a good source of antioxidants.

Effects of process variables on the texture

For a novel food product, it is necessary to study the impact of added ingredients on food quality attributes. Hardness or firmness is an important factor in the quality of breadsticks. The texture of dried *H. elongata* can be quite tough and processing is often required to make it more palatable. Common food processing methods such as boiling can lead to loss of phytochemicals (Cox *et al.*, 2011). To overcome the issues with the noticeable toughness of dried *H. elongata*, the dried seaweed was ground into a powder and was then incorporated into breadsticks. The model obtained for texture of the breadsticks was:

$$Z = 69.7308 - 0.0399788^* X_1 - 0.122297^* X_2 + 0.141849^* X_1^2 - 0.0002^* X_1^* X_2 + 0.0019626^* X_2^2 \quad \text{Eq. 5}$$

There was a significant (P < 0.05) influence of seaweed concentration, X_1, and the quadratic terms $X_1^*X_1$ (seaweed concentration × seaweed concentration) on the model (Table 3). However, there was no significant influence of white flour concentration (X_2) or the quadratic term $X_2^*X_2$ (seaweed concentration × seaweed concentration) or interaction term $X_1^*X_2$ (seaweed concentration × white flour concentration) on the model. The fit of the model was confirmed by a satisfactory R^2 value of 0.9981 which is very high. The response surface plot (Figure 1c) showed that the texture became harder with increasing seaweed concentration, but there were no major changes in hardness with increasing white flour concentration which was expected.

The hardness of the control breadsticks was calculated as 74.38 N/mm using an Instron texture analyser, and fortification of flour with seaweed at all levels (2.93 to 17.07%) significantly increased the hardness of the breadsticks (P < 0.05). Hardness

was maximized in the present study, when flour was replaced with 17.07% seaweed (108.84 N/mm). Prabhasankar *et al.* (2009a and 2009b) also found that adding seaweed to pasta (1 - 5%) increased the firmness of the product. Chang and Wu (2008) added 4 - 8% green seaweed to noodles and also found that there was an increase in the hardness with increasing seaweed concentration.

Effects of process variables on the color

Commonly *H. elongata* is dried and during the dehydration process, color darkens from brown to almost black (Cox *et al.*, 2012). Color is an important characteristic for baked products because together with texture and aroma, it contributes to consumer preference. It is dependant on physicochemical characteristic of the dough (water content, pH, reducing sugars and amino acid content) and on the operating conditions applied during baking (temperature, relative humidity, modes of heat transfer) (Esteller and Lannes, 2008). The consumer understanding of the expected color of baked goods is well known and this characteristic color would be expected with new baked products. The model obtained for color change of breadsticks with added seaweeds was:

$$Z = -0.562436 + 2.64694^* X_1 + 0.499152^* X_2 - 0.159474^* X_1^2 + 0.03885^* X_1{}^* X_2 - 0.0233189^* X_2^2 \quad \text{Eq. 6}$$

Color analysis of the breadsticks indicated that the linear factor of seaweed concentration (X_1) had an insignificant effect on the color of the breadsticks ($P > 0.05$) however the quadratic factors of seaweed concentration ($X_1{}^* X_1$) were significant ($P < 0.05$). X_2 (white flour concentration) also had a significant ($P < 0.05$) effect on the color of the breadsticks. There was no significant interaction of the quadratic term $X_2{}^* X_2$ (white flour concentration × white flour concentration) or interaction term $X_1{}^* X_2$ (seaweed concentration × white flour concentration) on the model ($P > 0.05$) and the R^2 value obtained was 0.7780. This indicated that both seaweed and white flour concentrations had some influence on the color of the breadsticks. This was further confirmed by the response surface plot (Figure 1d) as it had a spherical response surface which indicated that color change increased with increasing seaweed concentration but then gradually decreased, while white flour concentration also affected color change as it increased slightly with increasing flour concentration but then also decreased slightly. The color change of all samples was significantly different ($P < 0.05$) indicating that the different flour blends with varying concentrations of seaweed, white and wholemeal flour had a significant effect on the color

of the breadsticks. This was expected as the color of the seaweed is quite dark so varying the seaweed concentrations in the flour from 2.93 to 17.07% would obviously cause a difference in overall color of the baked breadsticks.

Optimization

Optimum conditions of seaweed and flour concentrations in breadsticks were determined to obtain maximum phytochemicals and enhance dietary fiber as the rational was to develop a functional food product. As the texture (hardness) and color of the breadsticks were acceptable throughout the ten experiments, they were not included as factors in the optimisation. These factors (texture and color) were sensorially evaluated by a sensory panel to determine acceptability. The second order polynomial models obtained in this study for TPC and DPPH responses were utilised in order to determine the specified optimum conditions. Optimum seaweed and white flour concentrations for maximising phytochemical constituents are depicted in Figure 2.

By applying the desirability function method (an approach for solving the problem of optimising several responses which have to be considered at the same time) the concentrations were obtained for the breadsticks with optimum phytochemical level. Multiple response optimisation indicated that phytochemicals in breadsticks could be maximized with 17.07% seaweed and 21.89% white flour concentrations in the overall flour. The response values predicted under these conditions by the multiple response optimisation were 142.75 mg GAE/100 g db for TPC and 64.58% for DPPH radical scavenging activity. A validation experiment was carried out by preparing breadsticks with the optimized dried seaweed and white flour concentrations. The phytochemical constituent contents were 138.25 mg GAE/100 g db for TPC and 65.01% for DPPH radical scavenging activity.
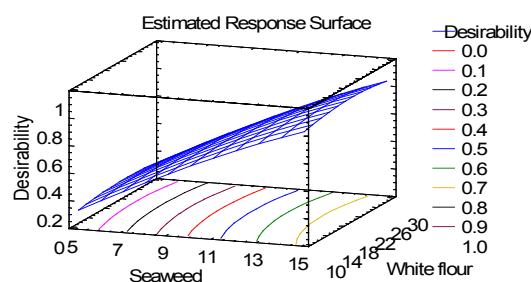

Figure 2. Response surface plot showing optimized effect of seaweed and white flour concentrations (%) to maximize phytochemical constituents of breadsticks

Total dietary fiber

In view of the therapeutic potential of dietary

fiber, more fiber incorporated food products are being developed. Figure 3 shows the total dietary fiber (TDF) content of the breadsticks. Dried seaweed contained 39.56% TDF, control breadsticks had 4.65% TDF and the seaweed breadsticks as optimized using RSM (17.07% seaweed added) contained 7.95% TDF which represents a 43.65% increase in the total dietary fiber when compared to breadsticks with no added seaweeds. Addition of seaweed significantly increased the TDF of the breadsticks as compared to the control ($P > 0.05$). These results are higher than those reported in the literature for final products containing seaweed. Prabhasankar *et al.* (2008) developed a seaweed pasta which had 4% fiber, but the amount of seaweed added was considerably less (2.5%). Cofrades *et al.* (2008) found that the addition of 5% *H. elongata* to meat systems only contributed 2.52% TDF to the final product. The same authors also found that the incorporation of *Porphyra umbilicalis* seaweeds at 5%, only fortified meat products with 1.77% fiber. The effect of enrichment of bread with rice bran fiber was studied by Hu *et al.* (2009) and addition of up to 6% rice bran fiber resulted in 4.98% TDF in the final product. Therefore, in the current study, the optimized breadsticks had a higher TDF in the final product (7.95%), this higher level would also be due to the fact that more seaweed could be added to the breadsticks then to the products in the other studies outlined in literature.
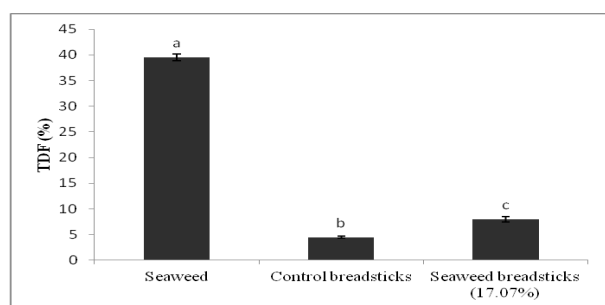

Figure 3. Total dietary fiber content of seaweed, control and seaweed breadsticks
Each value is presented as mean ± SD (n = 3).
Means above each bar with different letters (a-c) differ significantly ($P < 0.05$).

Sensory analysis

Table 6 summarises the sensory scores for aroma, appearance, texture, taste and overall acceptability of control and seaweed breadsticks. When developing functional bakery products, it is important to design a product with physiological effectiveness that will be accepted by consumers in terms of appearance, taste and texture (Siró *et al.*, 2008). The samples tested by the sensory panel in this study were the control (with no added seaweed), breadsticks with 10% of the flour replaced with seaweed (6.08% concentration of seaweed overall) and the optimized sample from the RSM study which would have the maximum level

of antioxidants (17.07% seaweed in overall flour blend or 10.33% seaweed in the final product).

Aroma, appearance, texture and taste were found to be significantly different to the control breadsticks ($P > 0.05$). Although there was a significant difference, the scores for each of the seaweed breadsticks were only slightly lower than that of the control, and all three breadsticks were at acceptable values suggesting potential incorporation of seaweeds in bakery products.

The results of the present study are promising as some food products with added fiber are often rated as unacceptable by sensory panels once they exceed a certain concentration. For example, Hu *et al.* (2008) found that the addition of rice bran fiber above 4% was unacceptable by consumers. Also, Prabhasankar *et al.* (2009) found that there was a significant difference in pasta with 10% replacement of semolina with seaweed as compared to the control ($P > 0.05$). This indicates that breadsticks are a good product for seaweed incorporation at high levels without affecting the overall quality of the product.

Table 6. Mean scores for aroma, appearance, texture and taste of the control and seaweed breadsticks

Breadsticks	Sensory attributes				
	Aroma	Appearance	Texture	Taste	Overall acceptability
Control	4.35±0.81a	4.40±0.50a	3.95±0.75a	3.8±0.61a	3.75±0.71a
10% seaweed	3.80±0.61b	3.30±0.92b	3.40±0.94b	3.50±0.68b	3.55±0.68b
17.07% seaweed	3.25±1.06c	3.30±0.92c	3.55±0.94c	2.75±0.85c	2.80±0.76c

Each value is presented as mean ± SD (n = 20).
Means within each column with different letters differ significantly ($P < 0.05$).

Conclusion

Response surface methodology using central composite design was demonstrated to be an effective technique for optimizing *H. elongata* and white flour concentrations for enhancement of phytochemical constituents in seaweed breadsticks. From the response surface plots, seaweed concentration was found to have the most significant effect on phytochemical content of the breadsticks. The high coefficients of determination of the variables at a 95% confidence level indicated that second order polynomial models could be employed to predict critical phytochemical parameters of breadsticks containing *H. elongata* along with texture and color. These breadsticks would provide the consumer with higher levels of dietary fiber (7.95%) and phytochemicals (TPC: 138.25 mg GAE/100 g db; DPPH: 65.01%) and have an appealing color and texture. There was a significant difference found in the sensory scores for seaweed breadsticks as compared to the control ($P > 0.05$), however all scores were at acceptable levels which is promising.

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